05250-05250

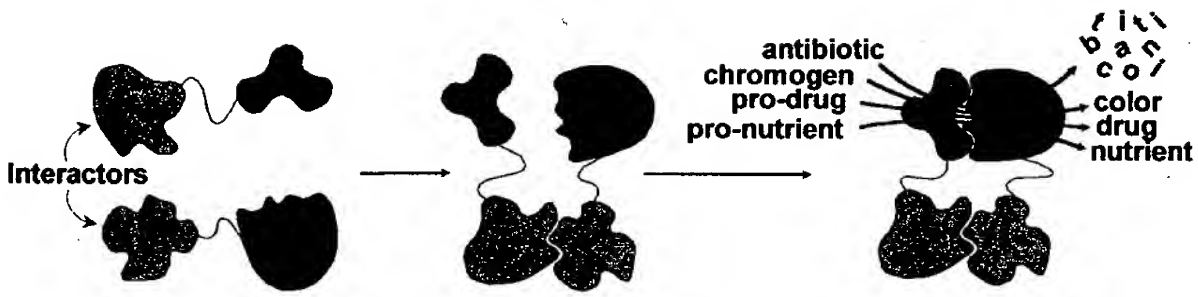


Figure 1

```
 76  cac cca gaa acg ctg gtg aaa gta aaa gat gct gaa gat cag ttg ggt
 26   H   P   E   T   L   V   K   V   K   D   A   E   D   Q   L   G

124  gca cga gtg ggt tac atc gaa ctg gat ctc aac agc ggt aag atc ctt
 42   A   R   V   G   Y   I   E   L   D   L   N | S   G   K   I   L

172  gag agt ttt cgc ccc gaa gaa cgt ttt cca atg atg agc act ttt aaa
 58   E   S   F   R   P   E | E   R   F   P   M   M   S   T   F   K

220  gtt ctg cta tgt ggc gcg gta tta tcc cgt att gac gcc ggg caa gag
 74   V   L   L   C   G   A   V   L   S   R   I   D   A   G   Q   E

268  caa ctc ggt cgc cgc ata cac tat tct cag aat gac ttg gtt gag tac
 90   Q   L   G   R   R   I   H   Y   S   Q | N   D   L   V   E   Y

316  tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa
106   S   P   V   T   E   K   H   L   T   D   G   M   T   V   R   E

364  tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta
122   L   C   S   A   A   I   T   M   S   D   N   T   A   A   N   L

412  ctt ctg aca acg atc gga gga ccg aag gag cta acc gct ttt ttg cac
138   L   L   T   T   I   G   G   P   K   E   L   T   A   F   L   H

460  aac atg ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg
154   N   M   G   D   H   V   T   R   L   D   R   W   E   P   E   L

508  aat gaa gcc ata cca aac gac gag cgt gac acc acg atg cct gta gca
170   N   E   A   I   P | N   D   E   R   D   T   T   M   P   V   A

556  atg gca aca acg ttg cgc aaa cta tta act ggc gaa cta ctt act cta
186   M   A   T   T   L   R   K   L   L   T   G   E | L   L   T   L

604  gct tcc cgg caa caa tta ata gac tgg atg gag gcg gat aaa gtt gca
202   A   S   R   Q   Q   L   I   D   W   M   E   A   D   K | V   A

652  gga cca ctt ctg cgc tcg gcc ctt ccg gct ggc tgg ttt att gct gat
218   G   P   L   L   R   S   A   L   P   A | G   W   F   I   A   D

700  aaa tct gga gcc ggt gag cgt ggg tct cgc ggt atc att gca gca ctg
234   K   S   G   A   G   E   R   G   S   R   G   I   I   A   A   L

748  ggg cca gat ggt aag ccc tcc cgt atc gta gtt atc tac acg acg ggg
250   G   P   D   G | K   P   S   R   I   V   V   I   Y   T   T   G

796  agt cag gca act atg gat gaa cga aat aga cag atc gct gag ata ggt
266   S   Q   A   T   M   D   E   R   N   R   Q   I   A   E   I   G

844  gcc tca ctg att aag cat tgg
282   A   S   L   I   K   H   W
```

Figure 2

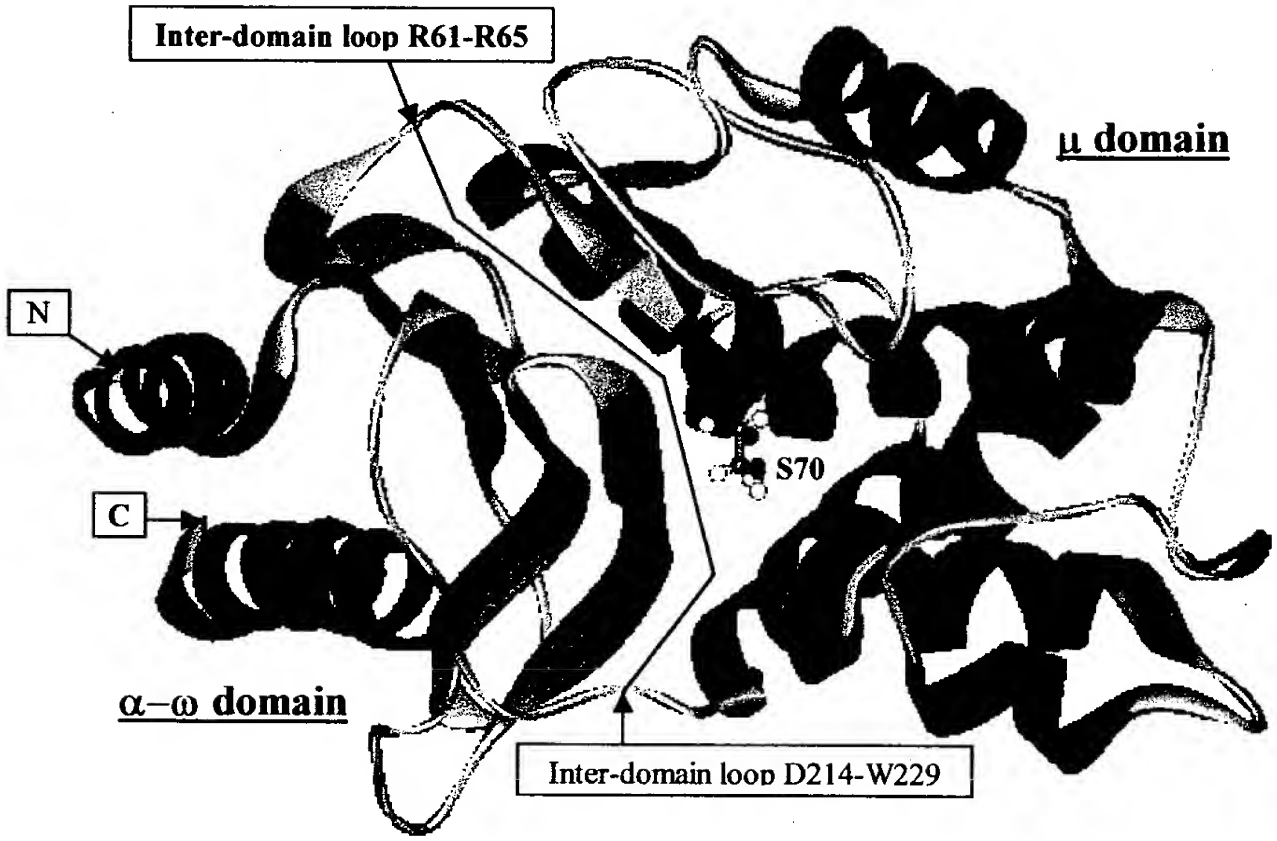

Inter-domain loop R61-R65
μ domain
N
S70
C
α–ω domain
Inter-domain loop D214-W229

Figure 3

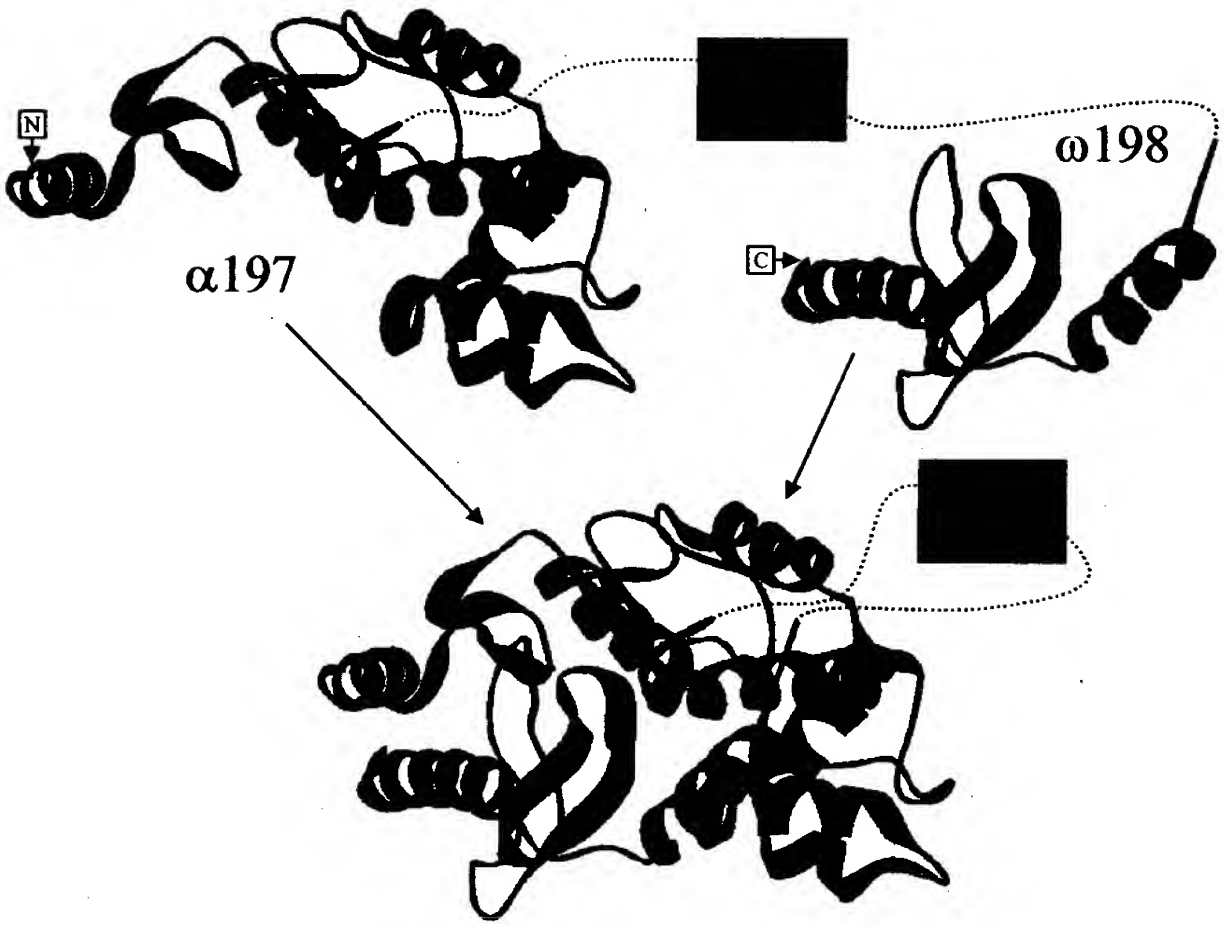
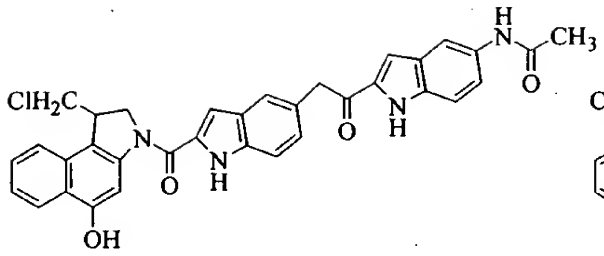
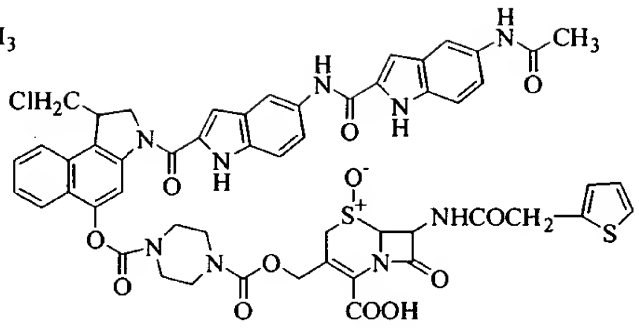


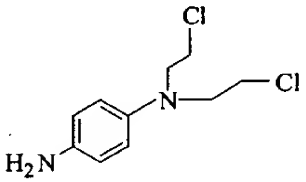
Figure 4



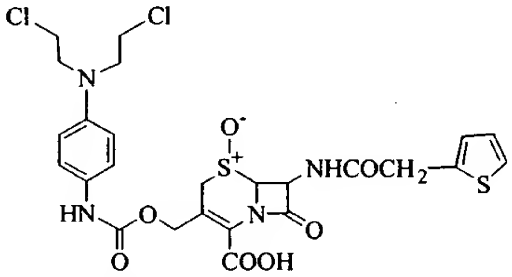
1. YW-200
IC_{50}: 0.01 nM



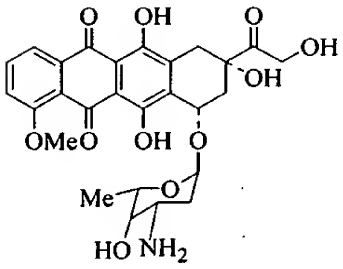
2. YW-285
IC_{50}: 10 nM



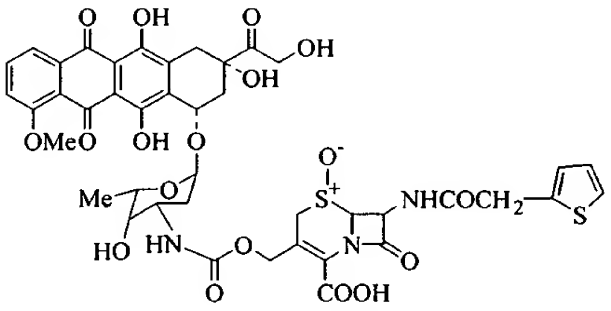
3. Aniline Mustard



4. Aniline Mustard Cephalosporin Prodrug

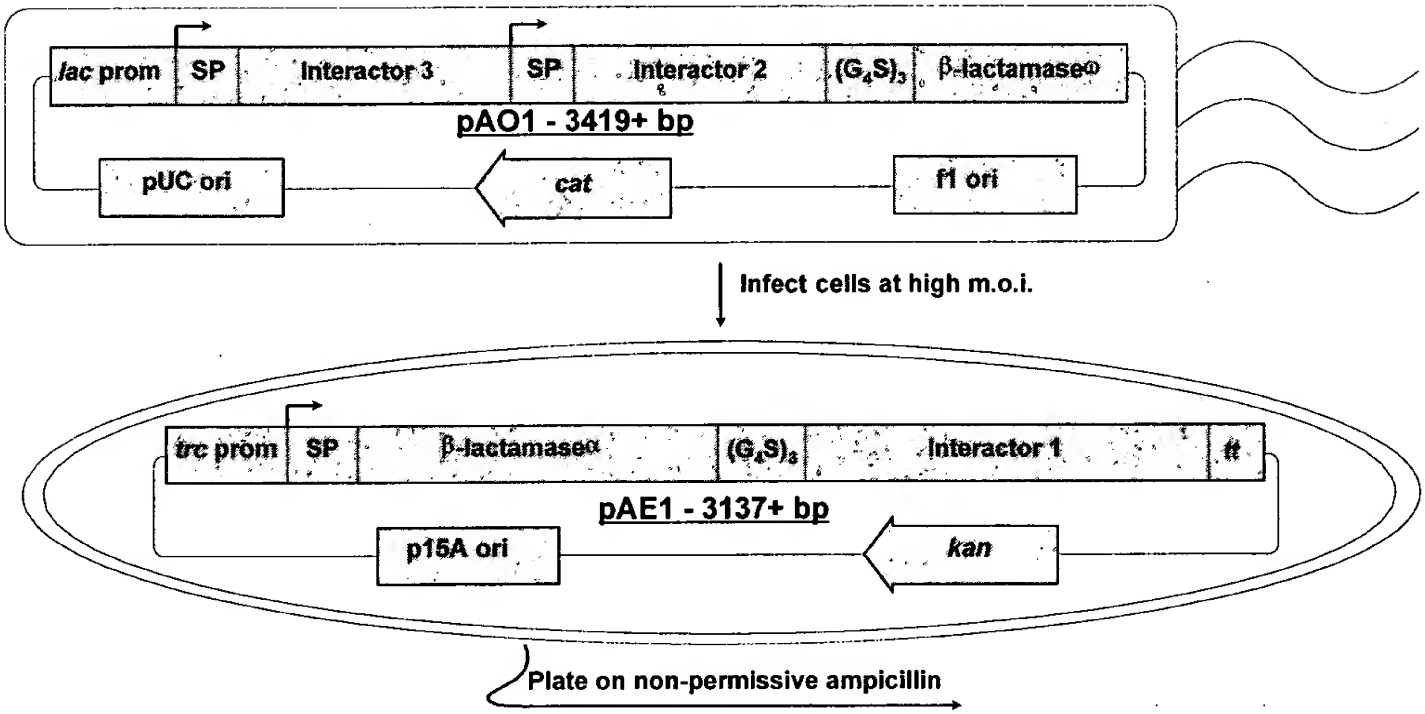


5. Doxorubicin



6. Doxorubicin Cephalosporin Prodrug

Figure 5



	Interactor 1	Interactor 2	Interactor 3
1.	Trxpep	scFv	none
2.	Trxpep	LC-CH1	none
3.	Trxpep	VL	none
4.	Trxpep	CD40	none
5.	Trxpep	Trxpep	CD40
6.	jun helix	scFv	CD40-fos helix
7.	jun helix	fos helix	none
8.	jun helix	fos helix	tripep-trx library
9.	ES library	ES library	none

Figure 6

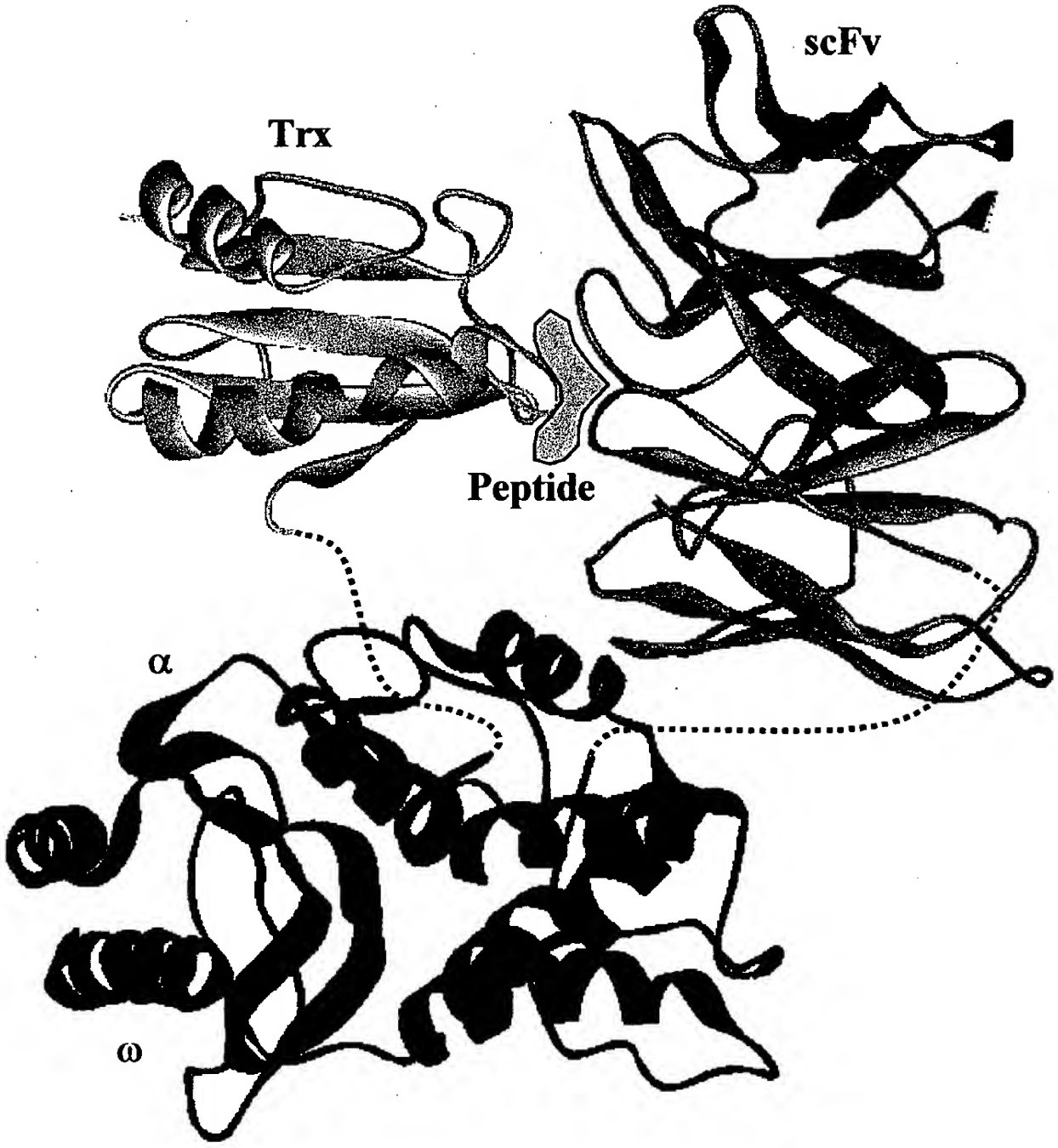


Figure 7

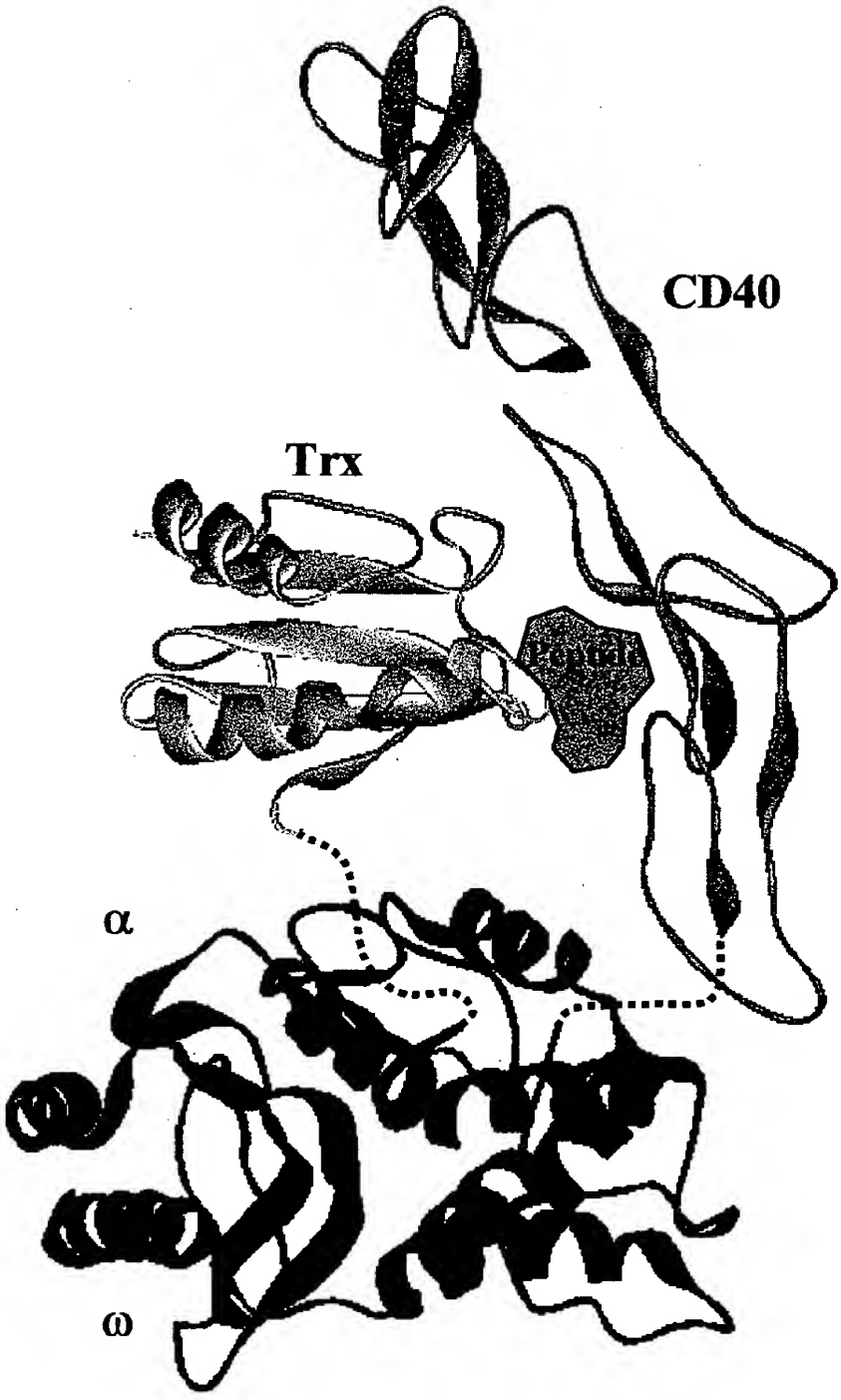


Figure 8

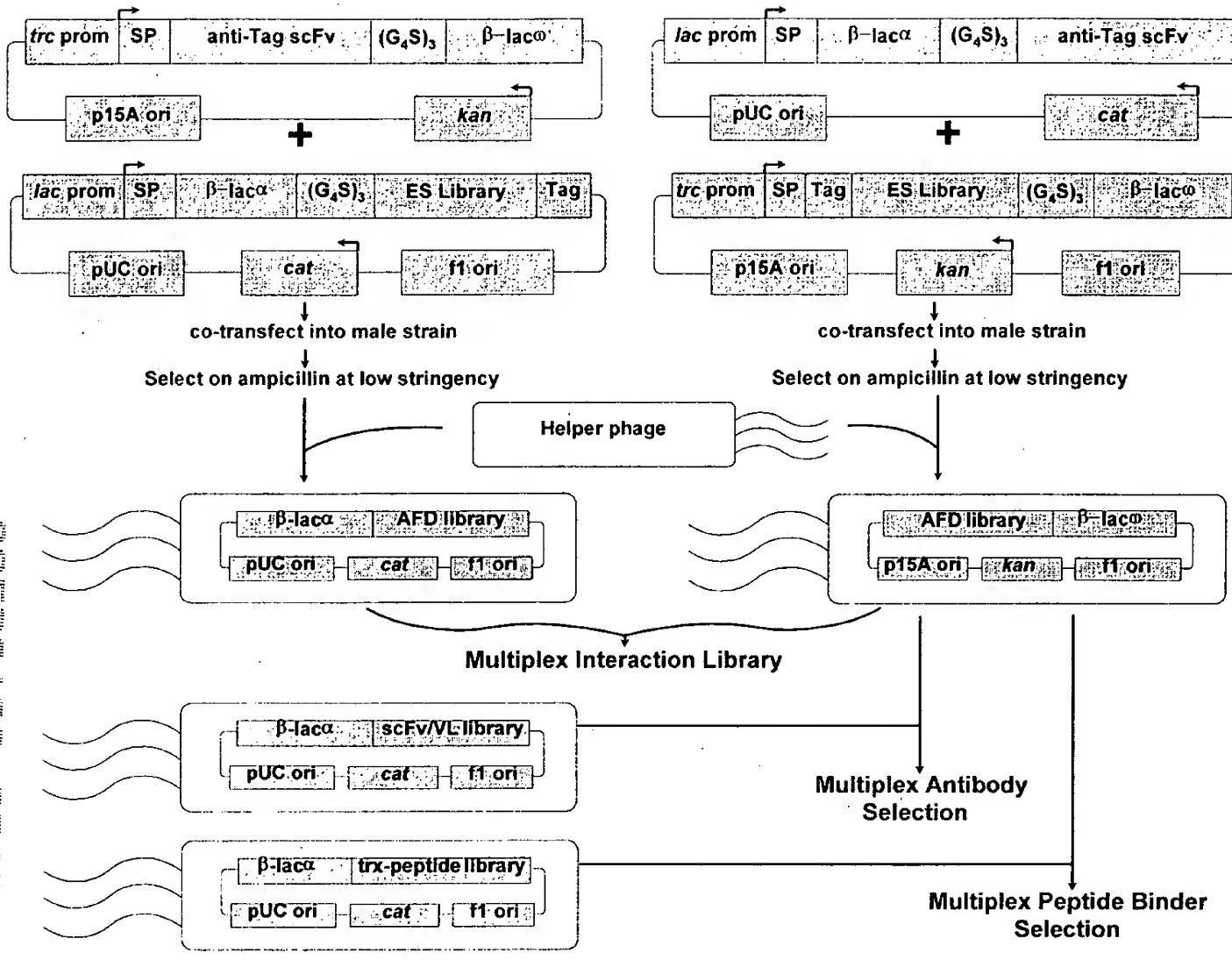

trc prom
SP
anti-Tag scFv
(G4S)3
β-lacω
p15A ori
kan
lac prom
SP
β-lacα
(G4S)3
ES Library
Tag
pUC ori
cat
f1 ori
co-transfect into male strain
Select on ampicillin at low stringency
lac prom
SP
β-lacα
(G4S)3
anti-Tag scFv
pUC ori
cat
trc prom
SP
Tag
ES Library
(G4S)3
β-lacω
p15A ori
kan
f1 ori
co-transfect into male strain
Select on ampicillin at low stringency
Helper phage
β-lacα
AFD library
pUC ori
cat
f1 ori
AFD library
β-lacω
p15A ori
kan
f1 ori
Multiplex Interaction Library
β-lacα
scFv/VL library
pUC ori
cat
f1 ori
Multiplex Antibody Selection
β-lacα
trx-peptide library
pUC ori
cat
f1 ori
Multiplex Peptide Binder Selection

Figure 9

```
                 ....,....1....,....2....,....3....,....4....,....5....,....6
         AA      |MGSAIEQDGLHAGSPAAWVERLFGYDWAQQTIGCSDAAVFRLSAQGRPVLFVKTDLSGAL|
         PHD sec |                 HHHHHHHH   EEEE      HHHEEE      EEEEE       |
subset:  SUB sec |LLL....LLLLLLL.HHHHHHH.LL......LLLLL.H......LLL.EEEEE.LLLLL.|
         Rel sec |98713346566989479999981552322125777546334224999378985177773|
access:  P_3 acc |eeebbeeeeeeee eeebbeebee ebbeeeebebbbbbbebeeeeeeebbbebeeeeee|
10st:    PHD acc |99700677777875797007606736007677060000006069796760006067777 7|

                 ....,....7....,....8....,....9....,....10...,....11...,....12
         AA      |NELQDEAARLSWLATTGVPCAAVLDVVTEAGRDWLLLGEVPGQDLLSSHLAPAEKVSIMA|
         PHD sec | HHHHHHHHHHHHHH      HHHHHHH     EEEEE                HHHHHHHHH|
subset:  SUB sec |..HHHHHHHHHHHHHLLLLL.............E.....LLLLL...LL.HHHHHHHHH|
         Rel sec |32779999999999659987232233212232235431027876542455359999999 9|
access:  P_3 acc |eebeeebe bbbbbeeebebbebbebbeeeeebbbbbebbebeebeee eeeeebbebbb|
10st:    PHD acc |77077606500000777060060060067776000007006077077757777700600 0|

                 ....,....13...,....14...,....15...,....16...,....17...,....18
         AA      |DAMRRLHTLDPATCPFDHQAKHRIERARTRMEAGLVDQDDLDEEHQGLAPAELFARLKAR|
         PHD sec |HHHHHH               HHHHHHHHHHHHHH        HHH     HHHHHHHHHH |
subset:  SUB sec |HHHHHH.LLLL.LLLLL...HHHHHHHHHHHH.LLLLLLL.....LLLLHHHHHHHHH.|
         Rel sec |99999838866468887332899999999997155558874221368678999999996 1|
access:  P_3 acc |ebbbebbebebeebbbeeebeeebeebeeebeebbb beebeeeeeeeebeebbeebeee|
10st:    PHD acc |70006007060770007770776077067607700050760776777607600760777 |

                 ....,....19...,....20...,....21...,....22...,....23...,....24
         AA      |MPDGEDLVVTHGDACLPNIMVENGRFSGFIDCGRLGVADRYQDIALATRDIAEELGGEWA|
         PHD sec |      EEEE        EEE    EEEEEE    HHHHHHHHHHHHHHHHHHHH HHHH|
subset:  SUB sec |LLLLLLEEEE.LLLLLL..E..LL.EEEEE.........HHHHHHHHHHHHHHHH.L.HHH|
         Rel sec |89899668862678866315318835788814311234478999999999975284999 |
access:  P_3 acc |eeeeeebbbbbbebbbbbbbbeebebbbbbbbb bbbbb bbbbbbbbeebeeebeee e|
10st:    PHD acc |67777700000060000000067060000000400000500000000660677077736 |

                 ....,....25...,....26...,....27...,....28...,....29...,....30
         AA      |DRFLVLYGIAAPDSQRIAFYRLLDEFF|
         PHD sec |HHHHHHH      HHHHHHHHHHHH  |
subset:  SUB sec |HHHHHHH.LLLLL..HHHHHHHHH.LL|
         Rel sec |99999973799883466757999835 9|
access:  P_3 acc |e bbeebbbee eeeebebbbbbeebb|
10st:    PHD acc |75006600076577760600000660 0|
```

Figure 10

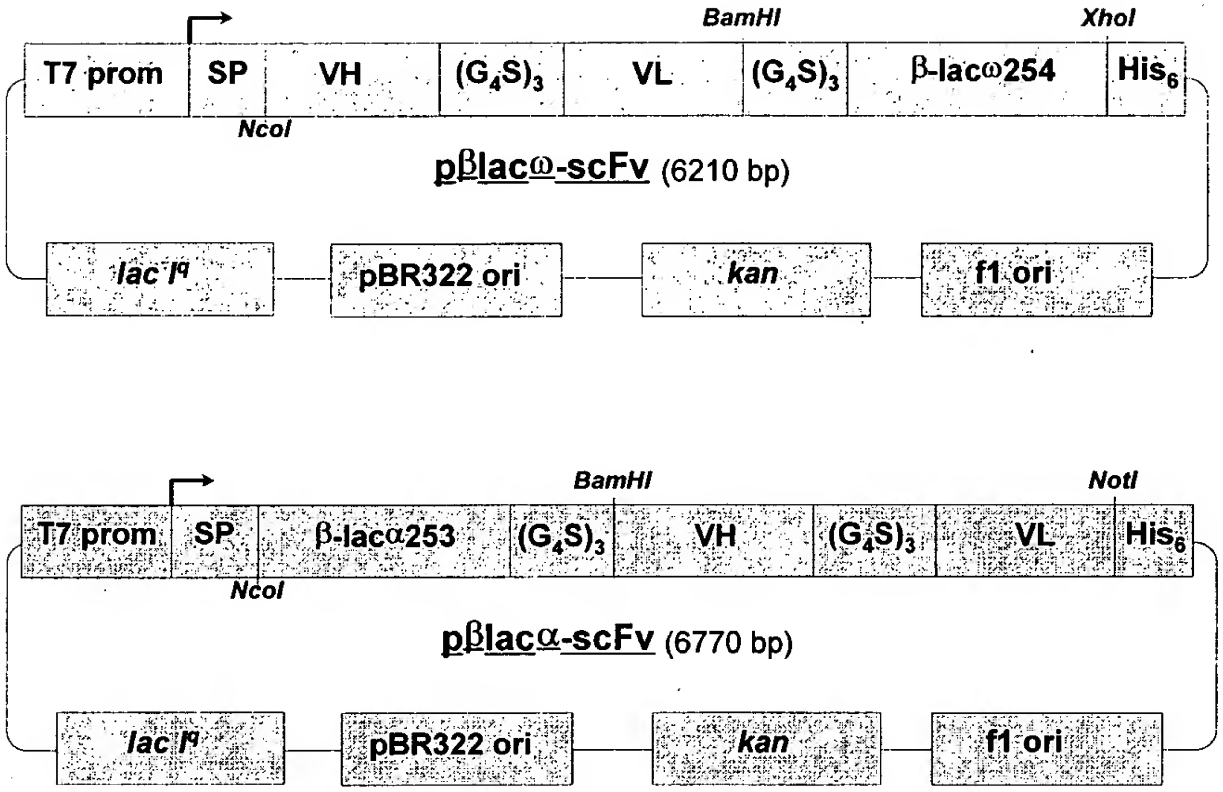

BamHI
XhoI
T7 prom
SP
VH
(G4S)3
VL
(G4S)3
β-lacω254
His6
NcoI
pβlacω-scFv (6210 bp)
lac Iq
pBR322 ori
kan
f1 ori
BamHI
NotI
T7 prom
SP
β-lacα253
(G4S)3
VH
(G4S)3
VL
His6
NcoI
pβlacα-scFv (6770 bp)
lac Iq
pBR322 ori
kan
f1 ori

Figure 11